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Comparison of Micro-Osteoperforation and Low Level Laser Therapy (LLLT) on Accelerated Tooth Movement – An Animal Study

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COMPARISON OF MICRO-OSTEOPERFORATION AND LOW LEVEL LASER THERAPY (LLLT) ON ACCELERATED TOOTH MOVEMENT – AN ANIMAL STUDY

ABELARDO DAYA ATTIE, D.M.D.

A Thesis Presented to the Faculty of the College of Dental Medicine of

Nova Southeastern University in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

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I certify that I am the sole author of this thesis, and that any assistance I received in its preparation has been fully acknowledged and disclosed in the thesis. I have cited any sources from which I used ideas, data, or words, and labeled as quotations any directly quoted phrases or passages, as well as providing proper documentation and citations. This thesis was prepared by me, specifically for the M.Sc.D. degree and for this assignment.

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Abelardo Daya Attie, D.M.D. Date

DEDICATION

To my parents, Fateh and Sonia, for all their support throughout my academic endeavors, for being my backbone, your unconditional love and for motivating me to always be my best.

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COMPARISON OF MICRO-OSTEOPERFORATION AND LOW LEVEL LASER THERAPY (LLLT) ON ACCELERATED TOOTH MOVEMENT – A RAT MODEL.

DEGREE DATE: December 2018

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Abstract

Introduction: Demand for orthodontic treatment is increasing amongst patients of all ages, including adults. Typically, a comprehensive orthodontic treatment requires two to three years of active tooth movement, which may not seem desirable for adults. Orthodontic appliances can impede proper oral hygiene and increase the risk of white spot lesions and caries. Other sequel of lengthy orthodontic treatment includes external apical root resorption, increased plaque index, increased level of dental caries and subsequent gingivitis, root resorption, gingival inflammation, and bone loss. Reduction of treatment time may reduce the risk of the undesirable sequel and increase the acceptance rate of

orthodontic treatment by adults. Some patients may be willing to pay more and undergo additional procedures in order to decrease treatment time and the side effects of orthodontic treatment. However, some of these procedures need a referral to a periodontist or an oral surgeon to be performed, they may be lengthy and involve an invasive surgical procedure in addition to adding expenses to the comprehensive orthodontic treatment. Therefore, there is a growing interest among orthodontists in adopting adjunctive procedures to accelerate tooth movement that are considered "minimally-invasive" to accelerate tooth movement. The available evidence to date suggests that both Low Level Laser Therapy (LLLT) and Micro osteo-perforations (MOP) have the potential to be adopted in routine clinical practice with no additional distress for the patient. However, despite the large majority of reports, no study has been conducted to compare the relative efficiency of the two techniques. This study aims to explore and compare the effects of two minimally invasive techniques to accelerate orthodontic tooth movement. **Methods:** 45 Sprague Dawley rats will be randomly divided into 3 groups of accelerated tooth movement with Propel® (n=15), LLLT (n=15), and control group without any intervention except orthodontic appliance (n=15). An orthodontic closed Nickel Titanium (NiTi) coil spring was extended from the central incisors to the maxillary first molars of each rat on the left side. The distance between the first molars and the central incisors was measured intraorally, using a digital caliper. Five rats from each group were euthanized at the 14 and 21 days. The histological observations and the rate of tooth movement elicited the differences between the two techniques and the control group. **Results**: Out of 45 rats, 40 remained healthy and demonstrated normal increased body weight throughout the 3-week experimental period. 5 rats were lost during the study due to hypothermia, since the

temperature of the procedure room was set too low. At the end of the study all appliances stayed in place without breakage and all experimental groups demonstrated movement of the tests molars at the end of the experimental periods. There were no statistically significant differences in the clinically measured distance of the central incisor to the test molars across the groups in either of the time points ($p < 0.000$), neither between groups ($p = 0.49$), or the interaction of groups by time ($p = 0.971$). A post hoc Tukey test showed that day-21 was significantly different from the baseline and also from 14 days to 21 days in all groups at $p < 0.01$. However, no difference was found between baseline and 14 days in control and propel groups (*p value:* 0.11 and 0.06). The evaluation of osteoclast numbers in two different time intervals (T1, T2) demonstrated the mean amount of 1.86, 2.00 and 9.57 for control, propel and LLLT groups, respectively. The evaluation of osteoclast numbers in two different time intervals (T1, T2) demonstrated the mean amount of 58.29, 60.57 and 209.86 for control, propel and LLLT groups, respectively. The amount of root resorption was evaluated based on the presence of root resorption on the external border of roots. It seems that the laser group demonstrated higher frequency and severity of root resorption compared to control and propel groups. **Conclusions**: The rate of tooth movement did not differ significantly between the propel and laser groups at three-time intervals (baseline, 14 days, and 21 days). The number of osteoclasts was significantly higher in the LLLT group compare to the propel and control groups at both time points. However, the number of osteoblasts was significantly higher only at 14 days in these groups. LLLT demonstrated more significant histological changes compared to propel and seems to have a more significant effect on acceleration of tooth movement in a rat model.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

1.1. Background

1.1.1. Biology and Biomechanics of Orthodontic Tooth Movement

Orthodontic tooth movement (OTM) happens as a result of a complicated interaction of cellular and molecular changes following insertion of a mechanical force on a tooth. The pressure-tension and piezoelectric theories explain the underlying biologic events of bone remodeling required for OTM (1, 2). When a mechanical force is applied to a tooth, the tooth is displaced within the periodontal ligament (PDL) space and compression and tension sites appear around the tooth. These sites experience alterations in blood flow, followed by a cascade of released biologic mediators that influence bone remodeling. At the tension side, blood flow is enhanced, followed by an increase in osteoblastic activity, bone deposition, and mineralization. The pressure-tension or biomechanic theory suggests that any distortion of PDL cells can stimulate production of prostaglandins and activate osteoblasts in the tension and osteoclasts in the pressure site. The presence of "positive" and "negative" tension in the PDL leads to bone deposition and resorption respectively (3).

According to piezoelectric or bending theory, bending of the bone, piezoelectric, or magnetic forces result in alteration of the ionic balance in the crystalline structure of the bone. This will lead to creation of electric currents, release of biologic mediators, and activations of multinucleated giant cells, fibroblasts, osteoblasts, and osteoclasts (2). Any distortion in the crystalline structure of bone can create piezoelectric forces. However, the

presence of nerve impulses and their subsequent action potentials are essential in creating larger electric fields to trigger the cellular response (4).

Orthodontic tooth movement occurs in three phases. The initial phase involves the movement of the tooth within the socket, followed by the bending of the alveolar bone and movement of the PDL fluids. After this initial movement, the teeth enter a lag phase in which the tissue oxygen levels alter due to blood vessel occlusion and hyalinization of the PDL. This leads to release of chemical inflammatory mediators (IL, TNFα, prostaglandins, MMPs, and integrins), which in turn result in extracellular matrix remodeling. On the third stage of OTM, the post-lag or acceleration phase, the hyalinization of tissue at the pressure side leads to movement of the tooth (5). Bone resorption and deposition occur at this phase, mainly through two inter-related signaling pathways, RANK/RANKL/Osteoprotegerin and RUNX2 (Figure 1) (6, 7).

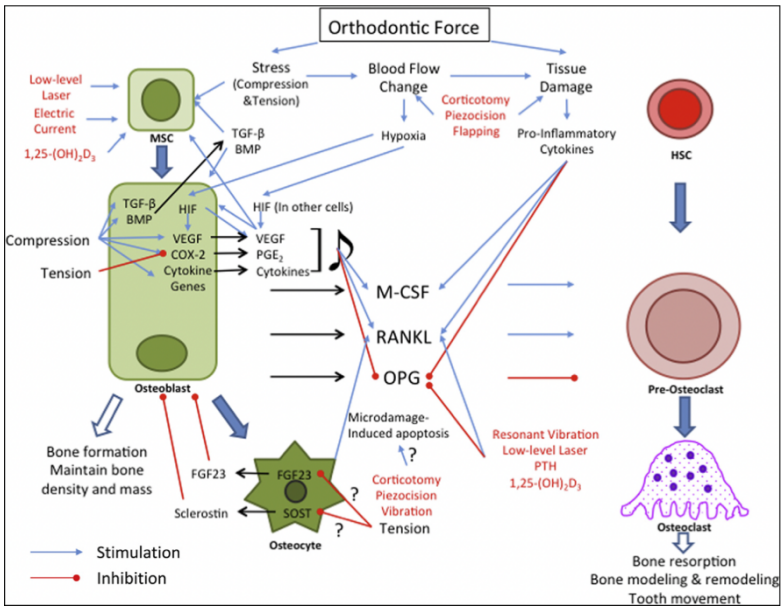

Figure 1.1. Outline of cellular and molecular mechanisms underlying accelerated orthodontic tooth movement. Red arrows: methods of accelerating orthodontic tooth movement and Blue arrows, inhibition; MSC, mesenchymal stem cell; HSC, hematopoietic stem cell; HIF, hypoxia inducible factor; FGF, fibroblast growth factor (Domínguez A, Gómez C, Palma JC. Effects of low-level laser therapy on orthodontics: rate of tooth movement, pain, and release of RANKL and OPG in GCF. Lasers in medical science. 2015;30(2):915-23).

1.1.2. Treatment Modalities to Accelerate Orthodontic Tooth Movement

Demand for orthodontic treatment is increasing amongst patients of all ages, including adults (8). Typically, a comprehensive orthodontic treatment requires two to three years of active tooth movement, which may not seem desirable for adults (9, 10). Orthodontic appliances can impede proper oral hygiene and increase the risk of white spot lesions and caries. Other sequel of lengthy orthodontic treatment include external apical root resorption (11), increased plaque index (12), increased level of dental caries and subsequent gingivitis (13), root resorption, gingival inflammation, and bone loss (14). Reduction of treatment time may reduce the risk of the undesirable sequel and increase the acceptance rate of orthodontic treatment by adults. Some patients may be willing to pay more and undergo additional procedures in order to decrease treatment time and the side effects of orthodontic treatment (15). As discussed above, there is an increase in demand and interest to develop and adopt adjunctive techniques to decrease the timing of orthodontic treatment and reduce the side effects.

On the other hand, as many cellular and inflammatory modulators play a role in OTM, many potential target areas are available to accelerate the rate of tooth movement (16, 17). The developed techniques includes the application of various chemical agents such as prostaglandins, relaxin, vitamin D, parathyroid hormone, growth hormone, corticotomies, micro-osteoperforations (Propel®) (14, 18, 19) and application of physical stimuli such as electromagnetic fields (20), piezocision (18, 19, 21, 22), vibration, electrical currents, and LLLT (18, 20, 21, 23-29) (Figure 1). The primary objective of all these techniques is to maximize the rate of tooth movement with minimal negative systemic and local side effects (30, 31).

1.1.3. Application of Lasers in Orthodontics

There is a growing interest in application of high and low intensity lasers in orthodontics (32). Lasers are commonly applied in orthodontics for enamel conditioning before bonding brackets, preventing the formation of white spot lesions (33, 34), gingival recontouring, exposure of impacted teeth, fiberotomy, and frenectomy (1, 35). To date, the low level laser therapy (LLLT) has been used for pain reduction after bonding of orthodontic appliance (25, 36, 37), pain management of temporomandibular joint disorders (38), increased bone turnover rate after rapid palatal expansion (39), and also, increased orthodontic tooth movement (1, 23, 26, 30, 39, 40). LLLT increases the RANKL levels in PDL, which increases osteoclastogenesis and consequently the rate of OTM. As a potential side effect, an increase in the intrapulpal temperature has been reported as a result of application of lasers to the tooth. However, this raise in temperature seems insignificant and does not appear to cause any harm to the pulpal tissue (41).

1.1.4. Low Level Laser Therapy and Orthodontic Tooth Movement

1.1.4.1. Mechanism of Action of Low Level Laser

Low level laser therapy (LLLT) or photobiomodulation involves the use of near infrared or low levels of red light for biological responses (42). As laser radiation does not increase the local tissue temperature by more than 1°C, it is referred to as “cold laser” or “low level laser” (43, 44). The mechanism of action of LLLT seems to be related to its effect at the molecular, cellular, and tissue levels. At the cellular level, the LLLT demonstrated an strong effect on mitochondria (45), whereby it enhances the expression of adenosine triphosphate (ATP) production and transcription factors (46, 47). These transcription factors are responsible for enhanced protein synthesis and modulation of

cytokines, inflammatory mediators, and growth factors (48). LLLT accelerates bone remodeling by increasing tissue vascularization and enhanced osteoid tissue formation (49), hence believed to be beneficial for acceleration of tooth movement. In vitro studies involving rat osteoclastogenesis cells have shown that laser irradiation induces differentiation and activation of osteoclasts (50-54) through enhanced expression of RANK, MMP-9, COX-2 (55), fibronectin (56), and collagen turn over. The TGF-B1 expression also was enhanced by LLLT in recent studies. TGF-B1 is an integral growth factor in differentiation and maintaining the function of osteoclasts (57, 58).

Fujita et al. (51) found a greater number of RANK and RANKL positive cells in laser treated groups compared to the non-irradiated and LED irradiated groups. An immunohistochemical evaluation demonstrated a higher degree of expression of RANKL in laser treated group in another study (59). However, the effect of LLLT on OPG was not as significant as it was on RANK (59, 60). OPG competes with RANK for binding to RANKL. Therefore, in the presence of LLLT, the ratio of OPG/RANK ratio is skewed in favor of RANK and therefore, there is a net increase in osteoclastic differentiation and activation after low level laser therapy (59).

In a recent study, Joes et al. (61) evaluated the effect of low level laser on IL-1B and prostaglandin cytokines. IL-I seems to be an essential cytokine to facilitate the maturation and activation of osteoclasts and initiate bone and root resorption (61, 62). IL-IB is a subtype of IL-1 cytokine, created mainly by monocytes and macrophages and responsible for prostaglandin E production. Prostaglandin E2 is responsible for bone turnover and the subsequent pain and discomfort experienced by orthodontic patients (63). IL-1B and PGE2 levels peak after LLLT and there was an statistically significant difference

in their level compared to the control group (61). However, in a clinical study, the LLLT did not show any significant effect on a pro-inflammatory cytokine, the IL-6 (20).

1.1.4.2. Effect of LLLT on Orthodontic Tooth Movement

Cruz et al. (47) were the first to investigate the effects of LLLT on orthodontic tooth movement in humans. They found a 34% increase in the rate of canine retraction in the LLLT group compared to the control group. Likewise, Soussa and Youssef et al. (48) found that LLLT significantly increased the rate of tooth movement of the upper and lower canines. Histologically, LLLT produced significantly higher number of osteoclasts and odontoblasts, as well as significantly greater deposition of collagen matrix at the pressure side (64). Other studies have demonstrated that laser irradiation promotes proliferation and maturation of osteoblasts (65), alters their mitochondrial activities, increases the production of growth factor and bone morphogenetic protein (BMP)(66), and increases enzyme activities an adenosine triphosphate (ATP) levels (67).

The findings regarding the acceleratory effects of LLLT on OTM are not consistent across the reported studies, perhaps due to the variation in experimental designs and a wide range of laser parameters and settings used, which make the interpretation of their results challenging (68). However, most of the studies conclude that LLLT can accelerate OTM within a given range of parameters. Energy density and application dose were recognized as the key determinants in eliciting the desired biological response (69, 70). This is best explained by "Arndt-schults" law, which states that very small doses of lasers fail to demonstrate any biological effect, moderate doses impede, and large doses are destructive for living systems (68). Various studies investigated the ideal parameter of low level laser and described the possible side effects or risks associated with this procedure (68). The

experimental doses varied from 1.89 to 702 Joules (J), and the effective range of energy density to accelerate OTM was reported to be between 5-54 joules/centimeter ^{2} (J/cm ^{2}) (46, 51, 60, 71-75).

A recent systematic review on application of LLLT on orthodontic tooth movement, reported that the diode laser was the most commonly selected source of LLLT. However, the wavelength, frequency, energy input, and hence the results were slightly different (76, 77). Shirazi et al. (76) demonstrated that LLLT can increase the rate of tooth movement 2.3-fold. On the contrary, Altan et al. (69) reported no difference between laser and control groups after application of high energy density. One possible explanation for their finding could be the application of higher energy density (54 J) in their study since the most effective range of LLLT for biomodulation is believed to be around 0.5–4 J/cm2.

Few randomized clinical trials have evaluated the clinical application of LLLT, as a non-invasive method to accelerate tooth movement (23, 28, 72, 78-81) (Table 2). A meta-analysis was conducted to evaluate the overall efficacy of LLLT on canine retraction. The results indicate that the retraction rate was significantly increased in LLLT group compared to control groups over the course of 21 days (78). Kim et al. (60) compared the effectiveness of high energy density laser therapy and corticision in accelerating orthodontic tooth movement. The major difference in LLLT setting was found to be related to the pulse mode used in their study compared to continuous mode used commonly in other studies. Interestingly, on the site that received both LLLT and corticision, there was a reduction in the velocity of tooth movement. However, the limited sample size in their study could have contributed to the inconsistencies in their findings compared to previous studies. As discussed before, the differences observed between the result of the existing

clinical studies could be attributed to different radiation doses employed (80). The radiation will have a cumulative effect over time (42). Therefore, significant increase in the rate of tooth movement is often seen in low level laser energy density (5-8 J/cm ^{2}) compared to high-level laser energy density (20-25 J/cm ^{2}) (28, 82). Further long-term studies are needed to determine the optimum laser wavelength, full delivery energy, repetition rate, dose and other properties to increase various tooth movement (42, 80).

1.1.5. Regional Acceleratory Phenomenon (RAP)

In 1959, Kole (83) pioneered the corticotomy technique to accelerate orthodontic tooth movement. He used the "wedge shaped crestal osteotomies" to facilitate OTM through the cortical bone (83). The definition of corticotomy is the surgical perforation or cuts through the cortical bone. The "regional acceleratory phenomenon" is commonly believed to be the underlying mechanism of accelerated OTM with corticotomy. This term was first used by Frost, an orthopedic surgeon who described the benefits of decortication to accelerate healing in bone injuries (84). According to Frost, corticotomy enhances bone inflammation, which in turn initiates bone demineralization. However, as the cuts are only made in the cortical bone with no injury to medullary bone, there will be no callus formation. Therefore, a significant advantage of corticotomy compared to osteotomy, is the lack of hyalinization in bone (85). In the presence of RAP, there is an earlier onset of osteoclastogenesis and therefore the overall turnover rate of bone is enhanced (86).

1.1.6. Application of Corticotomies and Osteo-perforations in Orthodontics

Application of corticotomies to accelerate orthodontic tooth movement dates back to over hundred years ago (87). These techniques involve surgical exposure and perforation

of the cortical bone with burs (88) and mallets (89) to stimulate the inflammatory process involved in bone remodeling and OTM through a "regional acceleratory phenomenon" (*i.e.*, the RAP effect) (90). However, these procedures are often invasive and require concomitant periodontal surgery to raise flaps. Consequently, such invasive techniques could result in several adverse effects on periodontium such as loss of attached gingiva (91), loss of interdental bone (91), development of new periodontal defects (92), and reduction in alveolar bone height (92). In addition, hematomas of the face and neck have been reported and attributed to these invasive techniques (93). Moreover, performing any surgical technique calls for referral to a periodontist or an oral surgeon. This may pose a significant financial burden on patients and possibly reduce patient acceptance of these techniques (94).

Alternative "non-invasive" approaches such as Piezocision evolved over the years to increase the efficacy and efficiency of these acceleratory OTM techniques (95, 96). The use of an ultrasonic cutting instrument eliminated the need to raise a flap and minimized the amount of injury to the surrounding soft tissues. Both corticotomy and piezocision techniques stimulate the RAP effect. Studies in human long bones showed a maintenance in the RAP effect of up to 6 to 24 months after injury (97). However, both techniques present risks of inflammation, bleeding, and infection of the surgical sites and a higher risk for root resorption (98). There seems to be a possibility of increased root injury and subsequently root resorption following piezocision due to the presence of surgical cuts in this technique (99). Additionally, these techniques appear to have a low acceptance rate among patients due to their cost and the surgical procedure involved (100).

To further reduce the amount of injury to soft tissues, a flapless creation of micro-osteoperforations of the cortical bone was introduced. The Micro-osteoperforation techniques (MOPs) increase the levels of inflammatory mediators and osteoclastic activities and consequently increase the rate of tooth movement. The most common technique involves the use of the Propel® (Propel Orthodontics, Ossining, NY), which is a device with a miniature adjustable screw at the end. The screw can be adjusted to allow for a desired depth of penetration into the bone. The penetrations pose minimal trauma to the soft tissues and do not require suturing. Typically, two to three penetrations are required mesial or distal to the tooth being moved (14, 18, 19). Propel® stimulates chemotaxis via injury to the cortex (14, 101). Micro-osteoperforation appears to be an efficient and safe procedure to accelerate orthodontic tooth movement and the pain and discomfort by this technique appeared to be no different than the control group with no intervention in a clinical study (20).

1.1.7. Orthodontic Tooth Movement in Rats

There are significant differences between the morphological and physiological aspects of the alveolar bone of rats and humans. However, rats are generally considered the best animal model to evaluate the orthodontic tooth movement (102-108). As rats lack canine and premolar teeth, they have ample space to move molars in a mesial direction (68). Ren et al. (101) have provided an established orthodontic tooth movement protocol for rats. Some of the practical advantages of using rats for studies of OTM include the availability of cellular and molecular biological techniques, and ease of histological preparation of samples (101). Furthermore, the rate of mandibular bone remodeling cycle at rat has been estimated to be between 10 and 30 days (109). This cycle may vary based

on the age of the subjects, as it happens more rapidly in younger animals (110). In addition, the remodeling rate of rat's alveolar bone (6 days) is significantly higher than human adult bone (10-120 days) (111). The most common shortcomings of using a rat model for evaluation of OTM are the distal drift of the molar and continuous eruption of the incisors (38).

1.2. Purpose of the Study

The available evidence to date suggests that both LLLT and MOP have the potential to be adopted in routine clinical practice with no additional distress for the patient (112). However, despite the large majority of reports, no study has been conducted to compare the relative efficiency of the two techniques. This study aims to explore and compare the effects of two minimally invasive techniques to accelerate orthodontic tooth movement. One of the main advantages of the LLLT and micro-osteoperforations, by use of Propel®, is that the orthodontists are able to perform these procedures in their office by themselves and there is no need for referral to a surgeon. LLLT seems to decrease pain and anxiety in orthodontic patients and improve cooperation and acceptance of treatment (113). Micro-perforations by Propel® is relatively non-invasive when compared to the traditional corticotomy and piezocision techniques since it does not require surgical incisions and is shown to be effective in accelerating orthodontic tooth movement in human (112). This study is the first to investigate and compare the relative effect of LLLT and MOP on the rate of tooth movement and their biological effect in a rat model. Each technique targets different pathways in the bone remodeling process required for OTM. Therefore, we expect to find differences in relation to their clinical and histological effects on bone. This study

will compare both techniques in order to guide clinicians to choose the appropriate technique for accelerated orthodontics.

1.3. Specific Aims

The overall goal of this study is to compare the effectiveness of two minimally invasive techniques, Low Level Laser Therapy (LLLT) and micro-osteoperforations (Propel®), to accelerate orthodontic tooth movement in a rat model. Specifically, the study aims to assess:

1. The rate of tooth movement in two different time intervals, comparing the micro-osteoperforations (Propel®) group to the control group.

   Hypothesis: Application of the micro-osteoperforations (Propel®) will increase the rate of orthodontic tooth movement.

2. The rate of tooth movement in two different time intervals, comparing the LLLT group to the control group.

   Hypothesis: Application of the LLLT will increase the rate of orthodontic tooth movement.

3. The histological changes within and across the micro-osteoperforations (Propel®) group in relation to the structural differences of the bone and presence of osteoclasts.

   Hypothesis: Application of Propel® will increase osteoclastic activity where applied.

4. The histological changes within and across the LLLT group in relation to the structural differences of the bone and presence of osteoclasts.

   Hypothesis: Application of LLLT will increase osteoclastic activity where applied.

Chapter 2: Materials and Methods

2.1. Sample Size Calculation and Experimental Animal Preparation

A total of 45 male Sprague Dawley rats (Charles River Laboratories, MA, USA) aged 9 weeks old (250-300g) were used in this study. The animal experimental protocol in the present study was approved by the Animal Care Committee (IACUC) at Nova Southeastern University (Approval No. 2018.06.SK1).

Sample size calculation was done based on a previous study by Yang et al. (11). This study compared the effects of micro-osteoperforation and corticision on the rate of orthodontic tooth movement in rats and found statistically significant differences when using 15 rats per group ($n=15$, $p<0.001$). Using this study as a guide, a standardized effect of 0.80, with a power of 80% and an alpha of 0.05, could be found by using a sample of size of 5 rats per group. Therefore, 15 rats per group and a total of 45 rats were included in the study to ensure adequate sample size.

All the rats were housed for two weeks prior to starting the study in the same place at a standardized room temperature and same light conditions and feeding with same diet and water to match the environmental factors in all groups. During the study, animals were kept in the animal center at NSU in cages of 2 at a constant standard of 12/12-hour light/dark environment and temperature of 22 ± 3 °C, 45 ± 10% humidity and provided with food and water ad libitum. Each rat was marked in each cage using a Sharpie® marker to mark the more proximal part of the tail with either a purple or a brown line to differentiate the animal per cage, then the tails were also marked with a second line for the procedure performed; red for propel, green for laser and blue for control. The health status of each rat was evaluated daily during the length of the study.

All samples were randomly divided into three different groups of control, LLLT and propel groups, including 15 animals in each (Figure 1).

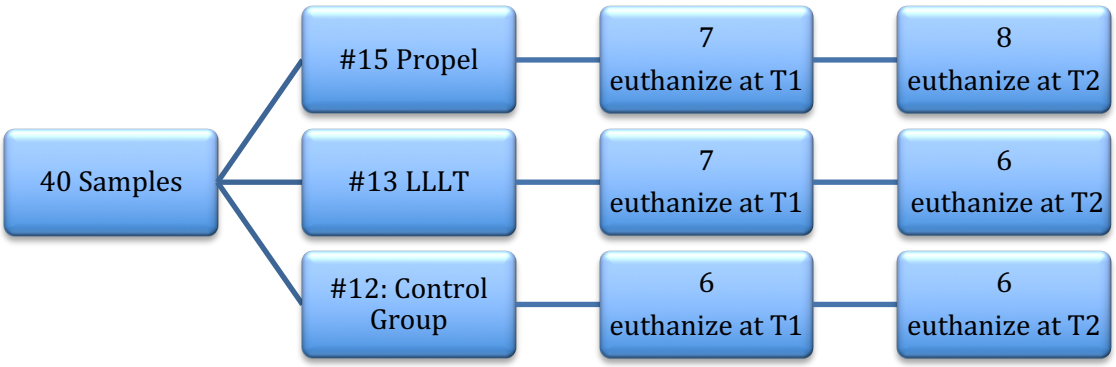

Figure 2.1. Flowchart of the sample distribution used in the study.
Animals were placed in Isoflurine chambers (1-3% Isoflourane and 100% O2) (Patterson, Veterinary, Colorado, USA) prior to general anesthesia injection for an average of 5 minutes. All animals received general anesthesia with xylazine (9.1 mg/kg) (AnaSed, Akorn, Inc., Lake Forest, IL) and ketamine (91 mg/kg) KetaVed (Vedco Inc., St. Joseph, MO, USA). An increased 25% of the dose was used only when needed.

2.2. Orthodontic Tooth Movement

A 25 g NiTi closing coil springs (Dentsply GAC International), was extended from the left first molar to the central incisors of each rat to apply 25gm of force at baseline. The NiTi coil spring was then ligated to the first molar and incisors with a 0.010-inch stainless steel ligature wire. The ligature wire (Dentsply GAC International) was inserted below the contact point of the first and second maxillary molars from the buccal side and pulled from the palatal side, using a Mathieu forceps. Only first molar was included in the posterior unit to maximize the protraction and create the larger differential anchorage between incisors and first molar.

A 25 g NiTi closing coil spring was passed through the ligature tie and the ligature was twisted until it wrapped around the tooth under its height of contour. The twisted end of the ligature tie was then secured with self-etch adhesive (Adper Prompt L-Pop Self-Etch Adhesive, 3M Espe, St. Paul, MN, USA) and flowable composite (Filtek Supreme Ultra, 3M Espe, St. Paul, MN, USA) to avoid unraveling and injury to the tissue. The coil was extended to the central incisors and secured by another ligature tie, which passed through the contact point of the two incisors (Figure 2 A, B). The ligature tie was secured by twisting until it wrapped the central incisors and was then bonded to the enamel with flowable composite. The maxillary incisors served as near absolute anchorage unit, as the roots are extremely long and curved (60, 71, 114, 115). The reason to encircle the ligature around the centrals were to increase the anchorage in the incisors area and to ligate the closed coil spring. The coils were stretched to the proper length to deliver a continuous 25 g force to the molars in a mesial direction. The amount of the delivered force was chosen based on the previous studies, suggesting that a 25gm force will promote tooth movement in rats (29-31 A).

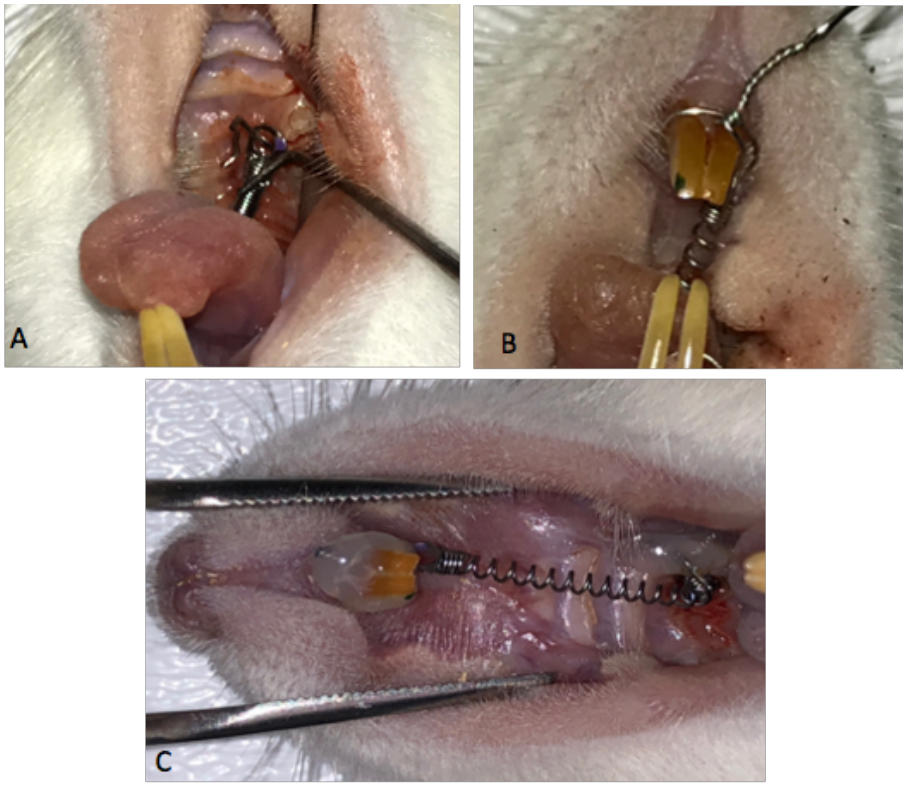

Figure 2.2. View of the orthodontic appliance in rats. **A**. A NiTi closing coil spring was passed through the ligature tie and the ligature was twisted until it wrapped around the tooth under its height of contour. The twisted end of the ligature tie was then secured with self-etch adhesive and flowable composite to avoid unraveling and injury to the tissue. **B.** The coil was extended to the central incisors and secured by another ligature tie, which passed through the contact point of the two incisors**. C.** Occlusal view of the appliance used for molar protraction.

2.3. Low Level Laser Therapy (LLLT)

For the LLLT group, the Gallium-Aluminium-Arsenide (Ga-Al-As) "Picasso" diode laser (AMD LASERS, classification IV4) was applied immediately after insertion of the coils. LLLT was applied on a total of 3 points including the mesiobuccal, the distobuccal and the palatal of the first maxillary molars (T0) (Figure 3). The location of LLLT application was determined as the 5 mm distance from the gingival margin in each of the three surface points measured by a periodontal probe. The points to be irradiated were at the level of mid root on both buccal and palatal sides. Irradiation was performed

by keeping the optical fiber tip (tip length - 5mm, Angle - 60º, fiber core diameter - 300µm) perpendicular to the surface, with light direct contact to the mucosa. The procedure was performed by the same researcher (A.D.) in an isolated room, using protective eyewear for the operator, and the research assistant.

The laser was delivered at a wavelength of 830 nm, in continuous mode of operation with the output power set at 0.9 W, 830 nm. These settings were chosen based on the latest recommendations by Fujita et el. (50). The laser was delivered for 4 seconds on each point for a total exposure of 12 seconds on each experimental side. Laser therapy was repeated for 3 consecutive days (once a day, from day 0-3), following Duan et al. study (52). In addition, the LLLT was done under anesthesia by Isoflurane to reduce the burden on the rats and better control of the location of irradiation.

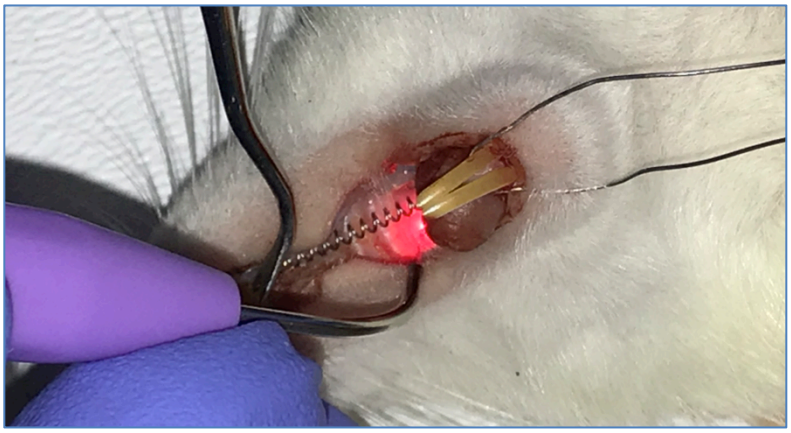

Figure 2.3. Low level laser therapy was applied on a total of 3 points including the mesiobuccal, the distobuccal and the palatal of the first maxillary molars for three consecutive days.

2.4. Micro-osteo Perforations (MOPs)

For the Propel® group, the Propel® device (Excellerator RT; Propel Orthodontics, NY, USA) was applied to a depth of 0.5mm and width of 0.25 mm at the distal and mesial

of the palatal aspect of the alveolar bone housing the first molar. The location of micro perforations was determined as the 5 mm from the gingival margin (measured by periodontal probe) in mesial and distal direction from the tooth line-angles of the first molar, with the distance of 5 mm (Figure 4).

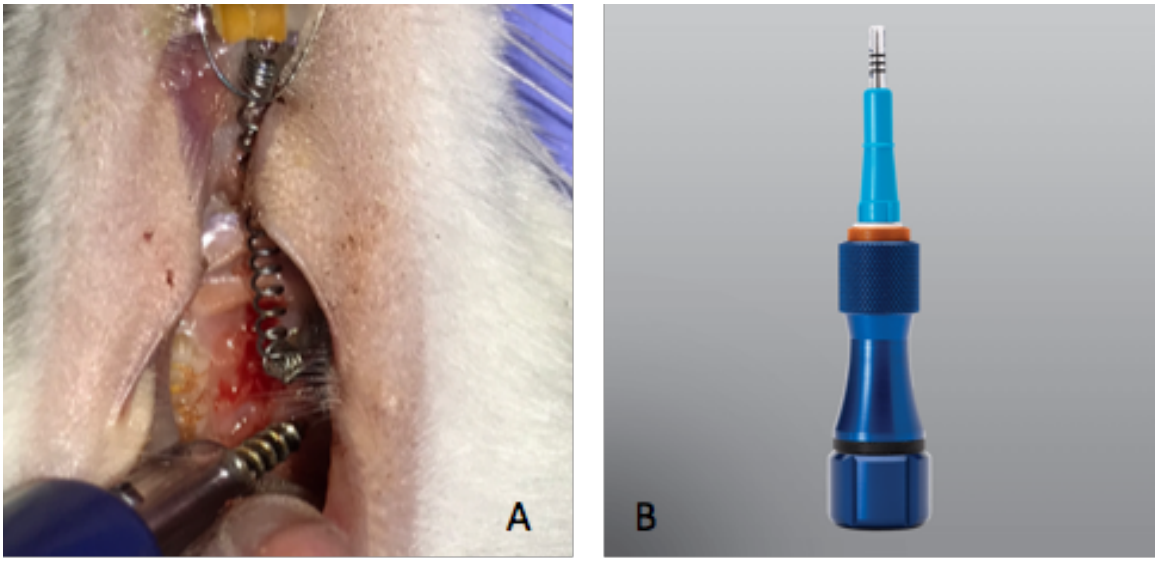

Figure 2.4. A. Two small MOPs were performed at a depth of 0.5mm and width of 0.25 mm at the distal and mesial of the palatal aspect of the left first molar. **B.** Handheld appliance designed by Propel Orthodontics (Excellerator RT; Propel Orthodontics, NY, USA) was used for performing MOPs.

2.5. Measurements

Tooth movement was evaluated clinically in the rats' mouth by use of a measuring device. A measuring device consisting of a 0.032 SS wire was bent to serve as a stop at the mesial surface of the cervical area of the maxillary first molar. This device was used to mark the distance between mesial of the left maxillary first molar to the palatal of the maxillary incisors, and then electronic digital calipers (Orthopli Corp, PA, USA) were used to measure the marked lines on the measuring device. The amount of tooth movement in

subjects were measured at three time frames of T0: Initial, T1: 2 weeks for samples euthanized in 14 days, and T2: 3 weeks for samples euthanized in 21 days under general inhalation of Isoflurane (Patterson, Veterinary, Colorado, USA). The distance (mm) and rate (mm/day) of tooth movement for each rat was recorded by subtracting the distance in millimeters measured at the baseline (T0) and the last time point (T1 or T2, based on the group). All measurements were done with the same investigator (A.D.). The appliances were checked daily, and adjustments were made per need to account for the continuous eruption of incisors in rats.

2.6. Tissue Preparation

To study the tissue response to applied techniques, 7-8 rats from each group were euthanized at two-time intervals (T1, T2). The maximum duration of the study was 21 days, which provides sufficient time to observe complete bone remodeling. The rats were euthanized using the CO2 smart box EUTHANEX (Euthanex EA-3100; Euthanex Corp, PA, 18043) for humane euthanasia. The maxillary arches were removed by incising the soft tissue with a #12 blade. The soft tissue was then reflected, using a 7A spatula until the bone was exposed. Slow speed motor and diamond disks were used to section the maxilla by first separating the zygomatic bone posteriorly. The sections were then extended anteriorly until a complete separation of the maxilla achieved without injuring the roots on the treatment side. (Figure 5).

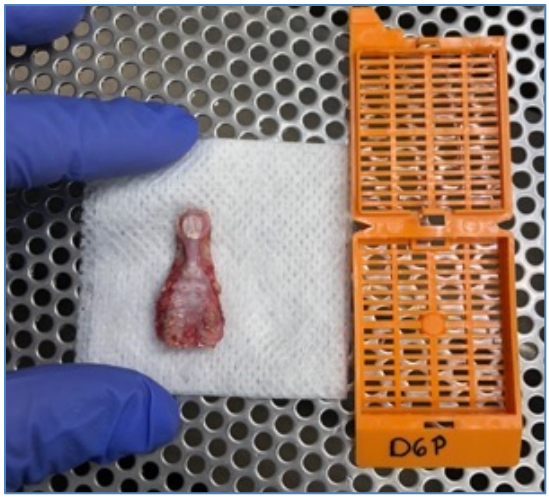

Figure 2.5. Sectioned maxillary arch before submerging in formalin 10%.

The sectioned maxilla was then placed in a cassette for specimens provided by the histology lab and submerged in formalin 10% to be sent for histological preparations. Samples were decalcified in 10% disodium ethylenediamine tetracetic acid (by 20% EDTA disodium salt, 200 gm, distilled H2O, 950 ml, 10N NaOH, ~50ml.

2.7. Histology

The specimens were dehydrated, embedded in paraffin and then sectioned, using a microtome. Each section was 5 microns thick and taken at 3 levels of roots adjacent to the compression and tension sites (based on the mesiodistal axis of the roots). Slides were ultimately prepared for hematoxylin and eosin (H&E). Three sections of slides at coronal, mid and apical levels were made for each side of specimens. After immunohistochemical TRAP staining, using the technique used by Carvalho-Filho et al.(89), a standardized grid (15 mm^2) was used for histomorphometric analysis of alveolar spongia and also, the osteoclast counts in the interradicular area of the test molars. This interradicular area was defined as the center of the boundaries of five roots of the first molar. To measure the

osteoclasts, slides were stained using tartrate-resistant acid phosphatase (TRAP) staining technique with available kit (Sigma Aldrich). Osteoclasts were identified as large multi-nucleated cells stained with eosin, containing round nuclei, which are present close to the bone surface. The root restorative area was measured by image analysis of the microscopic image that was magnified 10x to 40x when needed, based on the described method by Liu et al. (90). The data was expressed as square millimeters. For both the osteoclastic counts and root resorption area values, measurements were taken twice for each slide by two examiners and average number was then recorded.

2.8. Statistics

Statistical analysis was done using SPSS 16.0.1 (SPSS Inc, Chicago, III) software. In this experimental study, descriptive statistics (including the mean and standard deviation) for the rate of OTM in each group were calculated. Considering the normal distribution of data (Shapiro-Wilk test), the mean values of OTM were analyzed statistically and compared by paired t-test between the control and experimental groups.

Inter-group comparisons were done using analysis of variance with Bonferroni's correction (adjustment of P value for multiple groups). Intra-group comparisons will be done using linear general model for repeated measurements ANOVA was used to compare the amount of OTM at the experimental and control sides. A significance level of $p<0.05$ will be used for all data analysis.

· **Dependent variables:** Distance (mm, continuous) and Rate of teeth movement (mm/day, continuous), Osteoclast count (discrete).

· **Independent Variables:** Intervals of data collection, Types of interventions including Propel®, LLL in experimental side and no intervention at control sides.

· **Internal validity:** Selection threat- all rats were same type (acceptable age and weight range) and were randomly assigned to groups. Experimental and control sides also were randomly determined.

· **Instrumentation threat:** The amount of force was assessed using force gauge at baseline and the time of euthanize of the sample to make sure of consistent 25 g mesialization force on test molars during the study length.

· **Mortality:** The calculated sample size considered the possible number of loss of samples due to illnesses or deaths.

· **Experimenter Bias**: Randomization and data collectors were unaware of which group is receiving which treatment. First researcher organized cages with procedures and research assistants were unaware of procedure being performed.

· **Subject Bias**: Not applicable to rats.

Chapter 3. Results

Out of 45 rats, 40 remained healthy and demonstrated normal increased body weight throughout the 3-week experimental period. 5 rats were lost during the study due to a drop in body temperature after ketamine and xylazine were administered, since the temperature of the procedure room was set too low. To prevent this issue from recurring, the temperature of the remaining rats was maintained with use of a warm water bag until rats were sternal. All appliances stayed in place, without breakage or need for replacement. All experimental groups demonstrated movement of the test molars at the end of the experimental period.

3.1. Orthodontic Tooth Movement

We did not find statistically significant differences in the clinically measured distance of the central incisor to the test molars across the groups in either of the time points ($p < 0.000$). Neither did we find significant difference between groups ($p = 0.49$), or across different time points ($p = 0.971$). A post hoc Tukey test showed that day-21 was significantly different from baseline and also from 14 days to 21 days in all groups at $p < 0.01$. However, no difference was found between baseline and 14 days in control and propel groups (*p value:* 0.11 and 0.06) (Table 1).

Due to small sample size, the results were not statistically significant. However, when comparing the measurements of baseline (T0) and 21 days (T2) within and across the groups, a significant increase in the rate of tooth movement is noted in the LLLT group. The control group showed a mean movement of 3.14mm from T0 to T2. The LLLT group had a mean of 4.53mm and MOPs had a mean of 3.43mm of tooth movement comparing

the same time points. It appears that the LLLT accelerated the rate of tooth movement 8.1% faster than the control group and 7.21% than MOPs.

Table 3.1. Descriptive statistics for the amount of mesial movement of first molars within and between experimental and control groups in 2 time intervals.

Time = Baseline	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper 95% CI**
Control - Laser	-0.85	0.49	0.20	-1.81	0.11
Control - Propel	-0.73	0.47	0.27	-1.66	0.20
Laser - Propel	0.12	0.46	0.97	-0.79	1.03
Time = 14 days	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper 95% CI**
Control - Laser	0.49	0.67	0.75	-0.82	1.80
Control - Propel	-0.65	0.67	0.59	-1.96	0.66
Laser - Propel	-1.14	0.66	0.21	-2.44	0.16
Time = 21 Days	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper 95% CI**
Control - Laser	0.54	0.72	0.74	-0.87	1.94
Control - Propel	-0.45	0.67	0.78	-1.76	0.86
Laser - Propel	-0.98	0.67	0.31	-2.30	0.33
Group = Control	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper 95% CI**
Baseline - 14 days	1.20	0.58	0.11	-0.06	2.34
Baseline - 21 Days	3.14	0.61	<.0001	1.95	4.33
14 days - 21 Days	1.94	0.70	0.02	0.56	3.32
Group = Laser	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper 95% CI**
Baseline - 14 days	2.54	0.57	0.00	1.42	3.65
Baseline - 21 Days	4.53	0.60	<.0001	3.35	5.71
14 days - 21 Days	1.99	0.70	0.02	0.62	3.36
Group = Propel	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper 95% CI**
Baseline - 14 days	1.28	0.56	0.06	-0.19	2.37
Baseline - 21 Days	3.43	0.53	<.0001	2.39	4.47
14 days - 21 Days	2.15	0.65	0.00	0.87	3.42

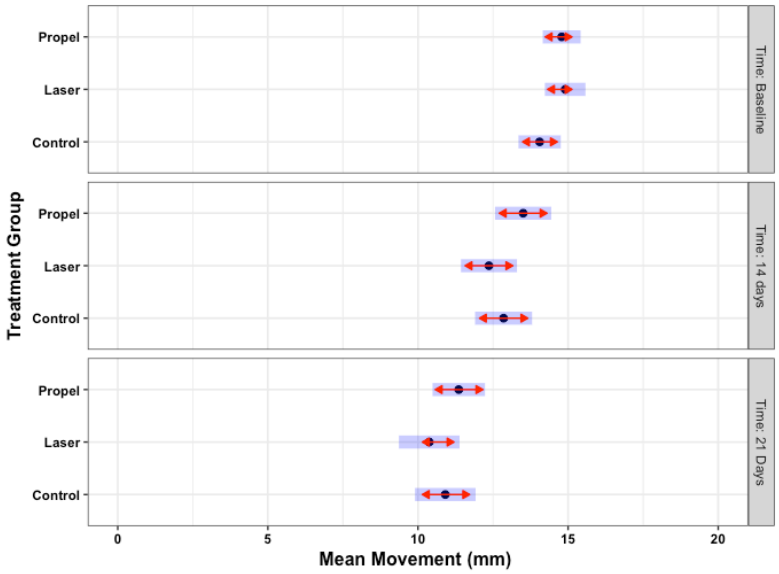

Figure 3.1. Tukey HSD pairwise results using Movement as the criterion

Note: The blue bars are confidence intervals for the means, and the red arrows are for the comparisons among them. If an arrow from one mean overlaps an arrow from another group, the difference is not significant.

3.2. Change in Histological Parameters

Histological results include the comparison of number of osteoclasts, osteoblasts and also presence of root resorption in different groups at two-time intervals.

3.2.1. Osteoclast Numbers

Table 3.2. Descriptive Statistics for osteoclast numbers in 0.25 mm^2 surface area after orthodontic tooth movement within the groups at two-time points (T1-T2).

Contrast	Difference	SE	p.value	Lower 95% CI	Upper (95% CI)
14 days – 21 days Control	-0.14	2.09	0.95	-4.24	3.95
14 days – 21 days Laser	0.57	2.09	0.79	-3.52	4.67

14 days – 21 days Propel	0.13	1.94	0.95	-3.68	3.93

The evaluation of osteoclast numbers in two different time intervals (T1, T2) demonstrated the mean amount of 1.86, 2.00 and 9.57 for control, propel and LLLT groups, respectively. At week 3, catabolic activity, measured by the mean number of osteoclast like cells, in compression side, decreased in all experimental (propel and laser) groups (Table 3).

Table 3.3. Descriptive Statistics for osteoclast numbers in 0.25 mm^2 surface area, after orthodontic tooth movement in each group.

	Day 14 (T1)			**Day 21 (T2)**			**Total**
	Osteoclast Number (post)			**Osteoclast Number (post)**			**Osteoclast Number (Total)**
	M ± SD	**Min**	**Max**	**M ± SD**	**Min**	**Max**	**M ± SD**
Control	1.86 ± 1.35	0.00	3.00	2.00 ± 2.45	0.00	6.00	1.92 ± 1.85
Propel	2.00 ± 2.16	0.00	6.00	1.93 ± 1.89	0.00	5.00	1.93 ± 1.94
LLLT	9.57 ± 6.45	3.00	19.00	9.31 ± 5.48	2.00	15.00	9.31 ± 5.78
Total	4.48 ± 5.30			4.05 ± 4.67			

According to the result of the fixed effects ANOVA model, the type of intervention had significant effect on the observed result (p value: 0.000). However, timing didn't demonstrate a significant role on the observed difference between the groups (p value: 0.877). As it is demonstrated in table 4,5 and Figure 2, the paired comparison of experimental groups with control group at both T1 and T2, demonstrated significant difference between LLLT groups with control groups (p value: 0.000). Furthermore, LLLT

groups demonstrated significant difference with propel (p value: 0.000). However, the differences between propel groups and control groups were not statistically significant (p value: 1.00).

As it is demonstrated in Tukey HSD pairwise results at figure 2, there was no overlap between LLLT osteoclast numbers and both control and propel groups, confirming the significant difference in the number of osteoclasts among these groups and both control and propel groups.

Table 3.4. Fixed-Effects ANOVA results using Osteoclasts as the criterion

Predictor	**Sum of Squares**	***df***	**Mean Square**	***F***	***p***	**partial η^2**	**partial η^2 90% CI [LL, UL]**
(Intercept)	780.10	1	780.10	55.33	.000		
Groups	477.20	2	238.60	16.92	.000	.49	[.26, .61]
Time	0.35	1	0.35	0.02	.877	.00	[.00, .04]
Groups x Time	0.84	2	0.42	0.03	.971	.00	[.00, 1.00]
Error	493.45	35	14.10				

Note. LL and UL represent the lower-limit and upper-limit of the partial η^2 confidence interval, respectively.

Table 3.5. Tukey HSD pairwise results using Osteoclasts as the criterion in two different time intervals (T1: 14 days, T2: 21 days).

Time = 14 days:					
Contrast	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper (95% CI)**
Control - Laser	-7.71	2.01	0.00	-11.65	-3.78
Control - Propel	-0.14	2.01	1.00	-4.08	3.79
Laser - Propel	7.57	2.01	0.00	3.64	11.51
Time = 21 Days:					
Contrast	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper (95% CI)**
Control - Laser	-7.00	2.17	0.01	-11.25	-2.75
Control - Propel	0.13	2.03	1.00	-3.85	4.10
Laser - Propel	7.13	2.03	0.00	3.15	11.10

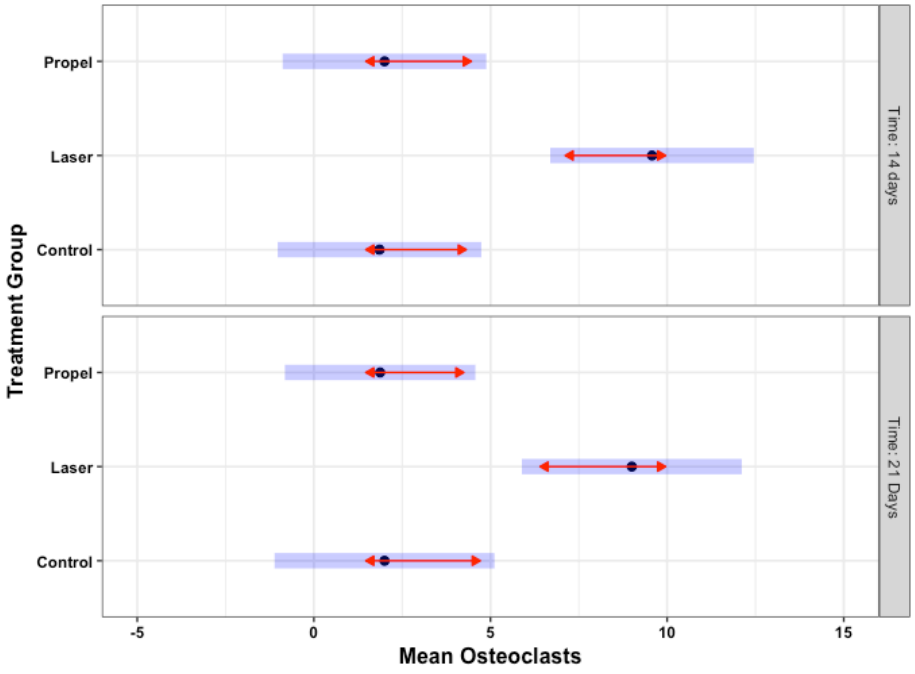

Figure 3.2. Tukey HSD pairwise results using Osteoclasts as the criterion in two different time intervals (T1: 14 days, T2: 21 days).

Note: The blue bars are confidence intervals for the EMMs, and the red arrows are for the comparisons among them. If an arrow from one mean overlaps an arrow from another group, the difference is not significant.

3.2.2. Osteoblast numbers

Table 3.6. Descriptive Statistics for osteoblast numbers in 0.25 mm^2 surface area after orthodontic tooth movement within the groups at two-time points (T1-T2).

Contrast	Difference	SE	p.value	Lower 95% CI	Upper (95% CI)
14 days – 21 days Control	-56.88	24.07	0.02	-104.06	-9.70
14 days – 21 days Laser	62.52	24.07	0.01	15.34	109.70
14 days – 21 days Propel	-26.55	22.39	0.24	-70.44	17.34

The evaluation of osteoclast numbers in two different time intervals (T1, T2) demonstrated the mean amount of 58.29, 60.57 and 209.86 for control, propel and LLLT groups, respectively. At week 3, anabolic activity, measured by the mean number of osteoblast like cells, in tension side, increased in all groups (Table 6).

Table 3.7. Descriptive Statistics for osteoblast numbers in 0.25 mm^2 surface area after orthodontic tooth movement in each group.

	Day 14 (T1)			**Day 21 (T2)**			**Total**
	Osteoblast Number (post)			Osteoblast Number (post)			Osteoblast Number (Total)
	M ± SD	Min	Max	M ± SD	Min	Max	M ± SD

Control	58.29 ± 29.33	28.00	98.00	115.17 ± 32.87	79.00	175.00	84.54 ± 41.85
Propel	60.57 ± 8.66	48.00	71.00	87.12 ± 11.48	76.00	105.00	74.73 ± 16.91
LLLT	209.86 ± 75.81	118.00	318.00	147.33 ± 61.81	93.00	228.00	181.00 ± 74.28
Total	109.57 ± 85.36			113.60 ± 44.65			

Same as osteoclasts number comparison, considering the result of the fixed effects ANOVA model, the type of intervention had significant effect on the observed result (p value: 0.000). However, timing didn't demonstrate to have significant role on the observed difference between the groups (p value: 0.611).

Table 3.8. Fixed-Effects ANOVA results using Osteoblasts as the criterion

Predictor	**Sum Of Squares**	***df***	**Mean Square**	***F***	***p***	**partial η^2**	**partial η^2 90% CI [LL, UL]**
(Intercept)	518820.29	1	518820.29	277.13	.000		
Groups	87272.73	2	43636.36	23.31	.000	.57	[.36, .67]
Time	493.02	1	493.02	0.26	.611	.01	[.00, .11]
Groups x Time	25073.11	2	12536.56	6.70	.003	.28	[.06, .43]
Error	65523.04	35	1872.09				

Note. LL and UL represent the lower-limit and upper-limit of the partial η^2 confidence interval, respectively.

As it is demonstrated in table 7,8 and Figure 3, the paired comparison of experimental groups with control group demonstrated significant difference at two-week

time point between LLLT groups with control groups (p value: 0.000). Furthermore, LLLT groups demonstrated significant difference with propel (p value: 0.000). However, the differences between propel groups and control groups were not statistically significant (p value: 0.99). The mean number of osteoblasts didn't have any statistically significant difference between the groups at 3-week time point (T2) (all p values were above the 0.005) (Table 8).

As it is demonstrated in Tukey HSD pairwise results at figure 3, there was no overlap between LLT osteoblast numbers and both control and propel groups at 14-day time point, confirming the significant difference in the number of osteoblasts among these groups and both control and propel groups. However, there was considerable overlap between the groups at T2, meaning no statistical significant difference among them at 21-days time point.

Table 3.9. Tukey HSD pairwise results using Osteoblasts as the criterion in two different time intervals (T1: 14 days, T2: 21 days).

Time = 14 days:					
Contrast	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper (95% CI)**
Control - Laser	-151.57	23.13	<.0001	-196.90	-106.24
Control - Propel	-2.29	23.13	0.99	-47.62	43.04
Laser - Propel	149.29	23.13	<.0001	103.96	194.62
Time = 21 Days:					
Contrast	**Difference**	**SE**	**p.value**	**Lower 95% CI**	**Upper (95% CI**
Control - Laser	-32.17	24.98	0.41	-81.13	16.80

Control - Propel	28.04	23.37	0.46	-17.76	73.84
Laser - Propel	60.21	23.37	0.04	14.41	106.01

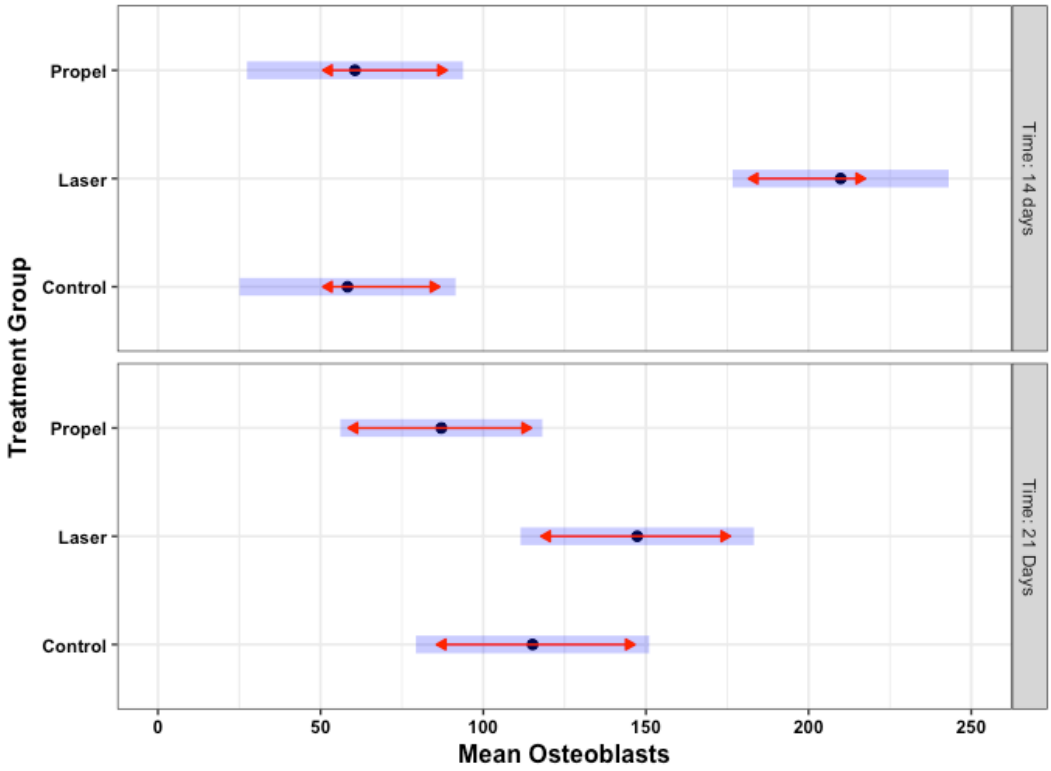

Figure 3.3. Tukey HSD pairwise results using Osteoblasts as the criterion in two different time intervals (T1: 14 days, T2: 21 days).

Note. The blue bars are confidence intervals for the EMMs, and the red arrows are for the comparisons among them. If an arrow from one mean overlaps an arrow from another group, the difference is not significant

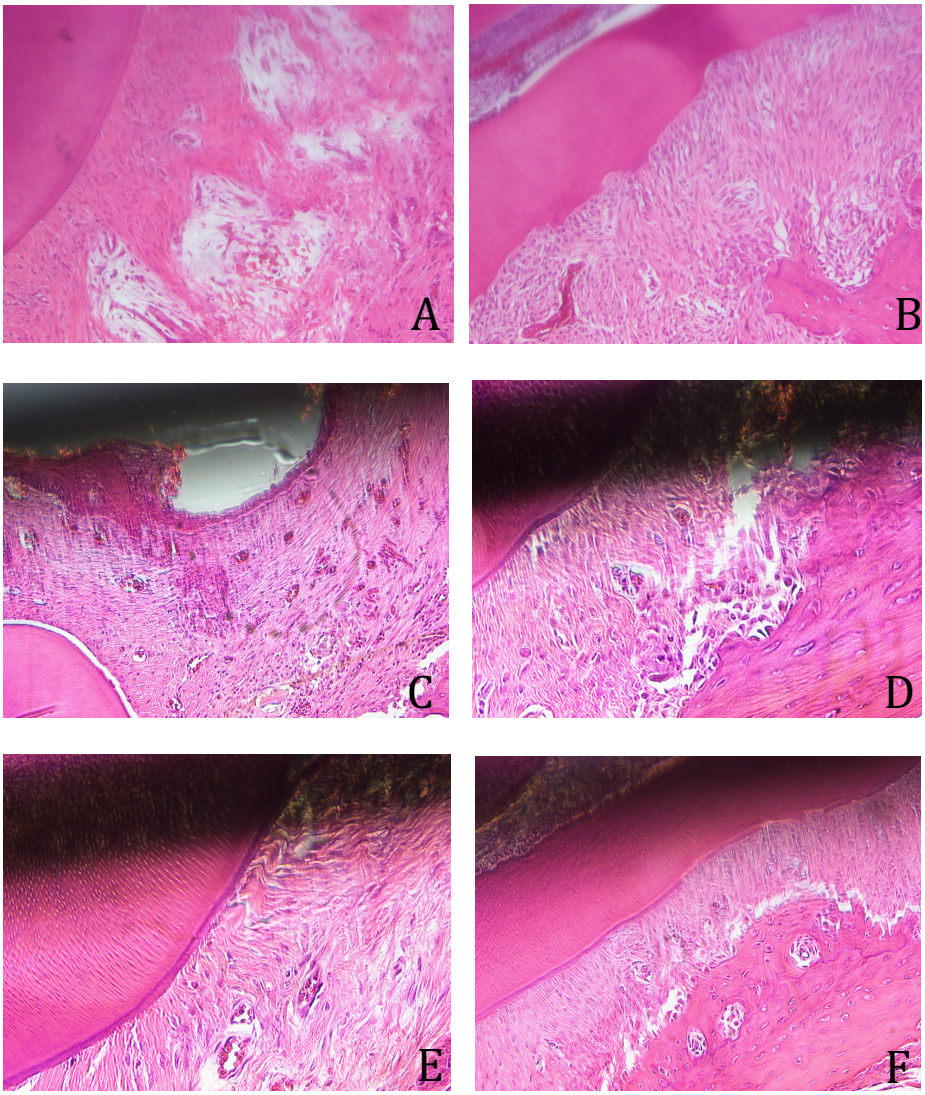

Figure 3.4. Histological osteoclast and osteoblast number in vertical sections at two-time point (T1, T2). (Control pics A and B; Propel pics C and D; LLLT pics E and F)

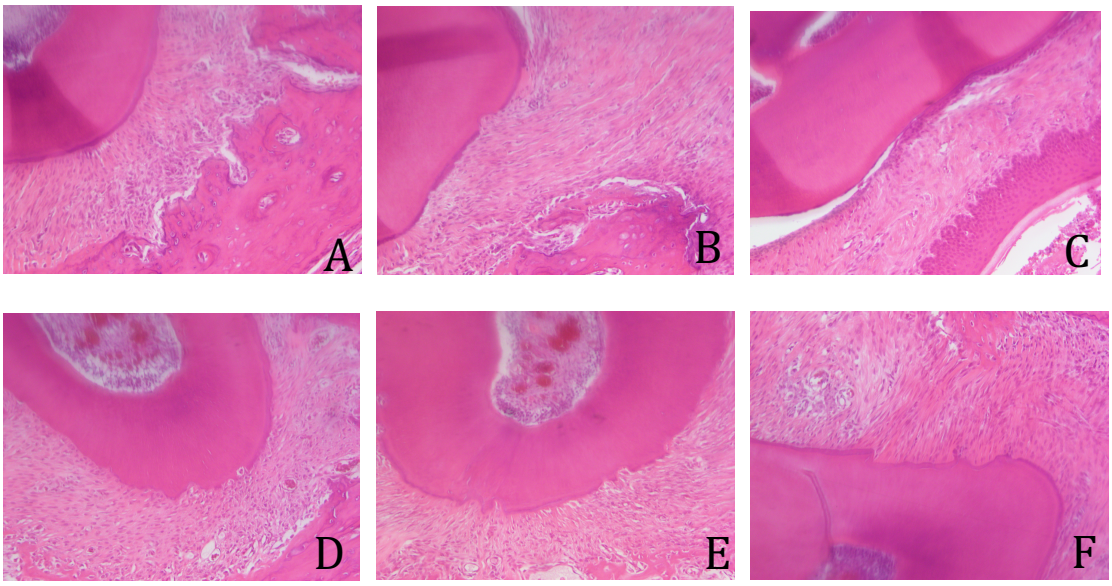

Figure 3.5. Histological cross-sections of control Group at each time point. (T1 pics A, B, C; T2 pics C, D, E.)

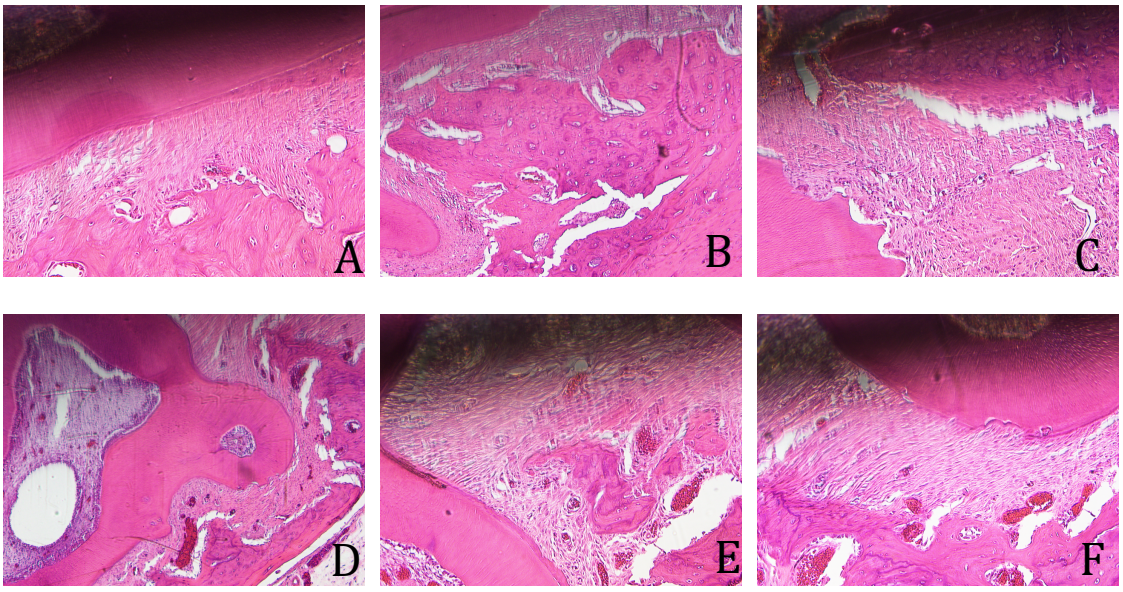

Figure 3.6. Histological cross-sections of Propel Group at each time point. (T1 pics A, B, C; T2 pics C, D, E.)

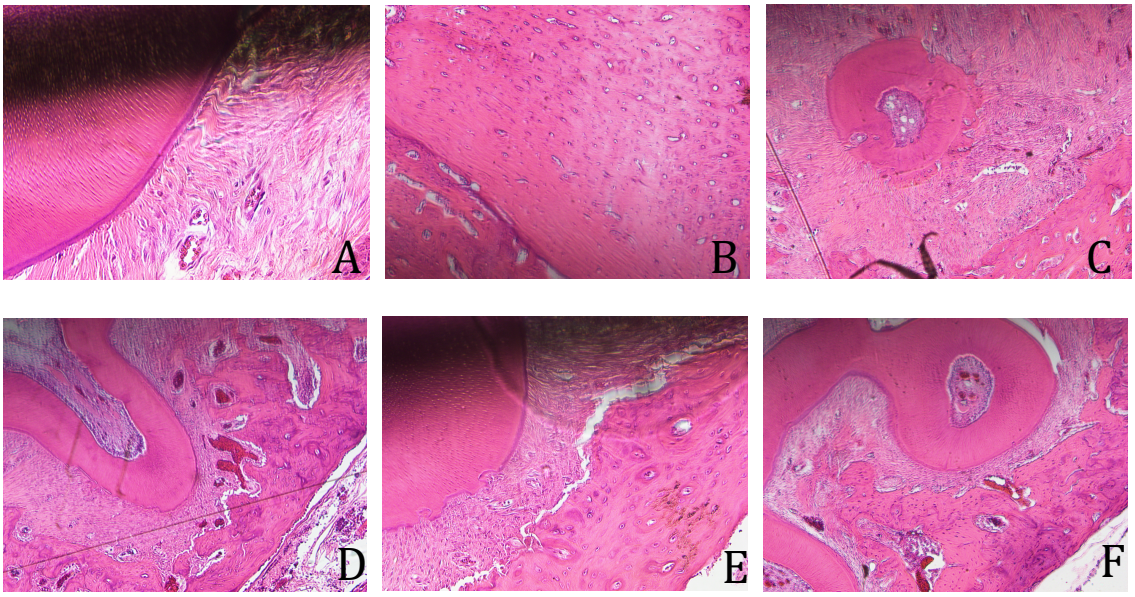

Figure 3.7. Histological cross-sections of laser Group at each time point. (T1 pics A, B, C; T2 pics C, D, E.)

3.3. Root Resorption Measurement

The amount of root resorption was evaluated based on the presence of root resorption on the external border of roots. Considering the longitudinal segments of the molar in histological preparations, the frequency and severity of root damage was evaluated based on 5 different levels. It seems that the laser group demonstrated higher frequency and severity of root resorption compared to control and propel groups. The overall score was 12 and 16 in T1 and T2 for laser group, compared to 6, 5 for control and 10 and 11 for propel groups, respectively. However, no statistical analysis could be performed due to the high frequency of 0 level in all groups.

Table 3.10. Descriptive Statistics for distribution of different root resorption levels after orthodontic tooth movement in two-time intervals. (0 no resorption – 5 significant resorption).

		0	**1**	**2**	**3**	**4**	**5**
14 days	Control	2 (0.29)	4 (0.57)	1 (0.14)	0 (0.00)	0 (0.00)	0 (0.00)
	Laser	3 (0.43)	1 (0.14)	1 (0.14)	0 (0.00)	0 (0.00)	2 (0.29)
	Propel	1 (0.14)	3 (0.43)	2 (0.29)	1 (0.14)	0 (0.00)	0 (0.00)
21 Days	Control	4 (0.67)	0 (0.00)	1 (0.17)	1 (0.17)	0 (0.00)	0 (0.00)
	Laser	1 (0.17)	1 (0.17)	1 (0.17)	0 (0.00)	2 (0.33)	1 (0.17)
	Propel	2 (0.25)	3 (0.38)	1 (0.13)	2 (0.25)	0 (0.00)	0 (0.00)

(0, 1: 0.5/8, 2: 1/8, 3: 2/8, 4: 3/8, 5: 4/8 of the proportion of total root)

Chapter 4. Discussion

In the present study, the effects of micro-perforation (propel) and LLLT on orthodontic tooth movement were investigated in a rat model. The LLLT group demonstrated significant effect only upon histological evaluations. There were no statistically significant differences in the rate of tooth movement across the groups at the two experimental time intervals. This contradicts the results of previous studies, using LLLT in rat model, which showed significant results on accelerating tooth movement (71, 75, 116). However, we observed a trend of increased tooth movement in both propel and laser groups. The average amount of tooth movement at 14 days ranged from a minimum of 1.2 mm in the control group to a maximum of 2.54 mm for LLLT group. We believe the use of micro-CT, for a more precise measurement of tooth movement, in a larger sample size could have increased the likelihood of achieving statistically significant differences across the groups. (75).

In previous studies, the amount of tooth movement at 14 days in control groups ranged from 0.26-0.6 mm. This study demonstrated the mean amount of 1.20 mm of movement in the control group. One explanation for this discrepancy could be the use of 25 g of force in this study compared to 10 gm in others. Another explanation is that we used direct intra-oral measurements of the distances of teeth instead of using indirect measurements via casts or application of micro CT. One justification for use of 25 g of force in our study was the possible need for reactivation of the coils if a 10 gm force was used in a rodent model. Previous studies show that such delicate coils may lose their activation over time (117). Another reason for application of 25 g of force in this study was to ensure occurrence of orthodontic root resorption. Former studies showed that application

of 10 g of force might enhance the orthodontic tooth movement, but root resorption may not occur (114).

Kim et al. (60), found that LLLT is as effective in acceleration of tooth movement as corticision. They evaluated the combined effect of corticision and LLLT, and found an inhibitory effect of combined treatment in rate of orthodontic tooth movement in beagle dogs (60, 174). It appeared that when used in combination, the LLLT shortened the RAP effect following corticision and resulted in an inhibitory effect on overall tooth movement (60). Another study by Suzuki et al. (114) combined the effect of LLLT and corticopuncture and reported the synergistic effect of these procedures on increasing the rate of tooth movement at both 14 and 21 days in a rat model. These conflicting results could be attributed to differences in the parameters of the two studies in use of corticopuncture and LLLT. Due to limited sample size, we were unable to evaluate the combined effect of these therapeutic procedures and only evaluated their individual effects on OTM.

4.1. Propel and Tooth Movement

The amount of orthodontic tooth movement in the propel group did not show statistically significant differences at T1 and T2, compared to the control group. The mean amount of movement was 1.28 mm in the propel group, as opposed to the reported mean amount of 1.39 mm in another study of micro-perforations (19). No significant difference was observed in the amount of tooth movement between propel and control groups at any time point throughout this experiment. This result is in contrast with a previous study comparing corticision and micro-perforation in a rat model (19). In the current study, micro-perforation was performed via manual application of the propel screw (width of 0.25

mm and depth of 0.5 mm) at the mesiopalatal and distopalatal areas of the root. In a study by Tsai (8), burs with the same width but less depth were used for perforations at the mesial of the first molar in three areas of root with 1mm distance. The number, depth, and location of micro-perforations could all contribute to differences observed in these studies. It is well documented that the application of RAP effect following corticision is not enough for the duration of orthodontic treatment and that these procedures need to be repeated for optimal results (60).

According to the previous studies, the most significant difference in the rate of OTM in a rat model can be seen in 2-weeks intervals (19). Therefore, we decided to eliminate the 1-week interval in this study to increase the sample size per group after the loss of five rats due to hypothermia. In contrast to previous studies, the amount of movement was increased significantly from T1 to T2 in the propel groups (p value: 0.00). Again, these differences could be attributed to the differences in the protocol used for micro-perforations in this study.

4.2. Laser and Tooth Movement

To date, many studies have evaluated the effect of low-level laser therapy on OTM in both animals and humans. Most studies reported that the application of LLLT stimulates the proliferation of osteoclasts, osteoblasts and fibroblasts and therefore increases the rate of tooth movement (61, 118). However, there is disagreement as to the role of fibroblasts in relation to accelerating soft tissue remodeling versus decreasing the amount of relapse and a possible increase in the resistance to tooth movement (119-121). This acceleration in tooth movement is possibly due to an increase in production of ATP and cytochrome C activation, through RANK/RANKL ligands (72). Moreover, the LLLT can enhance the

angiogenesis via an increase in release of nitrous oxide and therefore higher bone turn over (67, 75, 122). This will facilitate bone remodeling by helping the periodontium to eliminate hyalinized tissues following the application of orthodontic force and the resulting ischemia (123, 124).

The differences noted in the results of laser studies could be attributed to the differences in the laser characteristics used such as the wavelength, frequency, applied dosage, and energy density (76, 77). These studies indicate that in order to have a considerable bio-stimulatory effect by LLLT, the dosage and received energy per surface should be maintained within a range of 0.5-4 J/cm^2 (51, 69, 74, 125). Higher doses may have bio-inhibitory effect on the rate of tooth movement (116, 126) whereas lower doses may demonstrate no significant effect (127, 128). However, the heterogeneity of LLL settings in the previous studies makes it difficult to come up with an optimal recommended setting for this procedure. It has been shown that in humans, a higher energy density is needed to express the stimulatory effects on OTM (129) and that a low input cannot be fully compensated with an increase in the duration of LLLT (60).

4.3. Effect of Propel and Laser on Osteoblast and Osteoclast Activity and Orthodontic Tooth Movement

The histological examination showed that the mean number of osteoblasts in the propel group was higher than the control group at 14 days. However, this difference was not statistically significant (p value: 0.99). This number was not higher in the propel group at T2 (p value: 0.46). Previous studies in rats demonstrated the presence of RAP following a flapless micro-perforation within 2 weeks. Considering the limited duration of RAP in

rat models, repeating the micro-perforations might be indicated to re-induce the RAP effect.

In previous studies, it has been demonstrated that LLLT (830 nm) could enhance cellular proliferation, osteoblastic formation, and bone deposition (130). In the present study, the number of osteoblasts showed significant increase in the LLLT group compared to propel and control groups only at 14 days. No statistically significant difference was reported at 21 days (T2) among the groups based on the number of osteoblasts. Similar to a previous study, it seems that LLLT stimulates both osteoblastic and osteoclastic activity, with a more prolonged effect towards osteoclastic activity (131).

According to the available evidence, the differentiation and activation of osteoclasts may increase by LLLT through an increased expression of receptor activator of nuclear factor-jB (RANK), MMP-9, cathepsin K (50-54). In the current study, the mean number of observed osteoclasts in both time intervals was significantly higher in LLLT compared to control and propel groups. These results are in agreement with previous studies in which increased osteoclast surface was seen in the animals after LLLT (132).

On the other hand, in a very recent clinical study, it has been reported that the level of IL-1B and PGE2 was increased following LLLT (61). This could be attributed to the inflammatory reaction that is expected to happen when LLLT is performed. These cytokines may help the precursors to develop into osteoclasts and therefore increase bone resorption (61).

The amount of tooth movement in this study was comparable in both laser and propels groups. Therefore, considering the invasiveness of micro-perforation and the

associated pain and swelling, it can be concluded that laser therapy is less invasive and more efficient to accelerate OTM in a rat model.

4.4. LLLT Parameters

Among all the available acceleratory procedures, LLLT is the least invasive and most comfortable approach to be performed by orthodontists in their clinics (123). LLLT has demonstrated a significant increase in the rate of tooth movement (123). The only significant disadvantage of LLLT is the need for repeating the procedure in consecutive days, which may not be convenient for orthodontic patients who are usually seen every 4 weeks for routine adjustments.

The applied wavelength of LLLT in this study was 830 nm continuous mode, which was within the range of 630-850 nm reported by previous studies (42). As mentioned in a recent study, higher wavelengths increase the chance of LLL penetration to the periodontal ligament before it is absorbed by the surrounding bone (123). The parameters selected for LLLT in this study were a power of 900mW and radiation time of 12 S, applied every 3 consecutive days. This frequency was selected according to recommendation of Duan et al. (133) . However, this amount of power seems to be above the recommended range of 10-100 mW for LLLT in orthodontic tooth movement in human. This power was chosen based on the minimum amount of wattage of the commercially available diode laser in orthodontic offices. It was also less than the recommended threshold of 1000 mW, which demonstrated the dysplastic changes in the epithelium (117). This study used direct contact of fiber tip of the laser with specific points over the buccal and lingual root surfaces. However, the recent studies suggest application of circular movements instead of an static direct contact of LLL tip in order to prevent tissue ablation (117).

4.5. Limitations and Further Studies

This study was performed using a rat model. There is a considerable difference in dose-dependent tissue responses between humans and rat models, as some energy loss is expected to happen during LLL penetration through the soft tissue and bone and the characteristic of human periodontium is different from rat subjects (60). Additionally, for those studies using non-contact mode of LLLT, the application of power-detector device for measuring the energy density of the laser is suggested. However, in this study we used the direct contact mode of laser. Limitations in our knowledge of the optimal LLLT setting for human subjects, the differences in tissue structure, life cycle, and physiologic responses of tooth movements in humans, limit our abilities to interpret the result of this study in relation to application to humans (19). Further studies are required to confirm our result using different types of teeth (canine, premolar or incisors) and types of orthodontic tooth movement (retraction, protraction, intrusion or extrusion) in human subjects.

Chapter 5: Conclusions

The findings from this study support the following conclusions:

1) There were no statistically significant differences in the rate of tooth movement when comparing the groups. However, LLLT demonstrated 8% faster rate of tooth movement from baseline to 21 days compared to the control, and 7% compared to the MOPs group.
2) The rate of tooth movement did not differ significantly between the propel and control groups at the baseline, 14 days, and 21 days.
3) The number of osteoclasts was significantly higher in the LLLT group compared to the propel and control groups at 14 and 21 days. However, the number of osteoblasts was significantly higher only at 14 days.
4) The LLLT demonstrated significant histological changes compared to the propel and control groups. Therefore, it seems to have a more significant effect on acceleration of tooth movement.

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